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Genetic Aspects of Toxicity during Development

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The *Ah* locus in the mouse controls the induction of cytochrome P_1-450 and at least eleven associated monooxygenase activities. These enzyme systems metabolically potentiate and detoxify drugs, environmental pollutants, and other foreign chemicals, as well as numerous endogenous substrates. For certain substrates, it is known that cytochrome P_1-450 produces different reactive intermediates and products that other forms of P-450. Alleles at the *Ah* locus can be identified *in utero*. Developmental toxicity (in the form of stillborns, resorptions, and malformations of the fetus) by 3-methylcholanthrene and 7,12-dimethylbenz[a]anthracene given to the pregnant mother is associated with genetically mediated aromatic hydrocarbon responsiveness in C57BL/6N mice, compared with that in nonresponsive AKR/N mice. Acetaminiphen-produced hepatic necrosis is associated with glutathione depletion in the liver, covalent binding of metabolite(s) of the drug to cellular macromolecules, and P_1-450 induction controlled by the *Ah* locus. For reasons not known, the fetus and mice 10 days of age or less are relatively resistant to glutathione depletion and therefore hepatic necrosis by acetaminophen.

Introduction

The theme of this conference is evaluation of methods routinely used to assess developmental toxicity. Toxic effects on the developing embryo and fetus will be either environmental or genetic. This review will combine environmental and genetic factors, since our laboratory for the past 8 years has studied genetic differences in enzyme systems that metabolize drugs and other environmental agents.

Xenobiotics (foreign compounds) are, in general, hydrophobic chemicals that are metabolized by a group of enzymes known collectively as the cytochrome P-450-mediated monooxygenases (*1-5*). These membrane-bound enzyme systems are known to metabolize: polycyclic aromatic hydrocarbons such as benzo[a]pyrene (ubiquitous in city smog, cigarette smoke, and charcoal-cooked foods) and biphenyl; halogenated hydrocarbons such as polychlorinated biphenyls, insecticides, and ingredients in soaps and deodorants; strong mutagens such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and nitrosamines; aminoazo dyes and diazo compounds; *N*-acetylarylamines and nitrofurans; numerous aromatic amines (found in hair dyes), nitro aromatics, and heterocyclics; epoxides; carbamates; alkyl halides; safrole derivatives; certain fungal toxins and antibiotics; many of the chemotherapeutic agents used to treat human cancer; most drugs; both endogenous and synthetic steroids; and other endogenous compounds such as biogenic amines, indoles, thyroxine, and fatty acids. These enzyme systems may metabolically potentiate the detrimental effects of an inert parent compound by converting it to a reactive or toxic intermediate [benzo [a]pyrene is an example (*6,7*)] or may detoxify a reactive parent compound to an inactive product [*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine is an example (*8*)]. By "reactive" intermediate or parent compound, we mean an alkylating or arylating agent (usually an electrophile) capable of random damage to critical cellular macromolecules, thereby leading to toxic effects, mutation and cancer, or toxicity during development (i.e., birth defects).

The delicate balance in each tissue between enzymes which potentiate and those which detoxify highly reactive intermediates is being increasing-

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ly appreciated. This balance may be effectively altered by differences in genetics, age, hormonal or nutritional balance, circadian rhythmicity, and enzyme stimulation (induction) or inhibition due to drug-drug interactions. A myriad of alternative pathways, illustrated in the simple scheme shown in Figure 1, typifies the means by which xenobiotics are metabolized in the body.

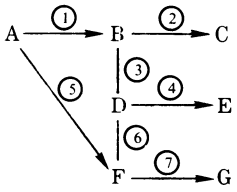

FIGURE 1.

If compound A, for example, causes toxicity, any factor increasing enzyme 1 or 5 would decrease the steady-state level of compound A and therefore decrease its toxic effects. If compound A requires potentiation to reactive intermediate B, any factor increasing enzyme 2,3, or 5 or decreasing enzyme 1 would decrease the steady-state level of the reactive intermediate and therefore decrease its toxic effects. In either event, if other more distant enzymes such as 2, 4, or 7 were rate-limiting for the overall pathway, a factor changing the level of such an enzyme could be most important in affecting the steady-state level of compound A or B.

Aryl Hydrocarbon Hydroxylase "Activity"

Evidence from this laboratory was presented (*9*) five years ago for a single gene difference between C57BL/6N (B6) and DBA/2N (D2) inbred mouse strains in the induction of a monooxygenase activity, hepatic aryl hydrocarbon hydroxylase (AHH), and newly formed cytochrome P_1-450 by 3-methylcholanthrene (MC) treatment. Figure 2 illustrates the AHH assay *in vitro*. Figure 3 shows the extent of hepatic AHH induction by MC in B6 and D2 mice as a function of age. The basal enzyme activity in either mouse strain in detectable during the last 5 days of gestation and increases rapidly during the first week postnatally. The phenomenon of this "physiological" increase in hepatic monooxygenase activity occurring immediately postpartum is not well understood but presumably is similar to the appearance of enzymes metabolizing, for example,

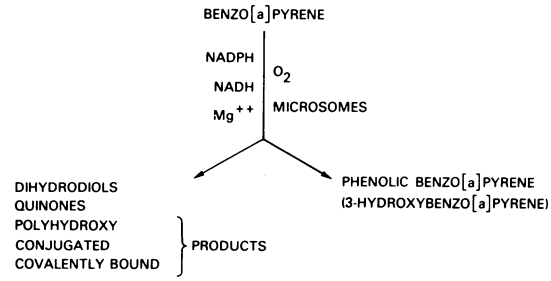

FIGURE 2. Current concept of the aryl hydrocarbon (benzo[a] pyrene) hydroxylase (AHH) "activity." The substrate benzo[a]pyrene is oxygenated to an arene oxide which rearranges nonenzymically to the phenol. Oxygenation via direct oxygen insertion (*1*) is a second possible mechanism. Other oxygenated derivatives of benzo[a]pyrene, including dihydrodiols and quinones, are not measured by this assay (*4,10*).

chloramphenicol or bilirubin. Related clinical entities are the Gray Baby Syndrome (*12*) and neonatal hyperbilirubinemia, in which the newborn and especially the premature have not yet developed the necessary microsomal enzymes for metabolizing chloramphenicol and bilirubin, respectively. MC transplacentally induces fetal hepatic AHH to detectable levels 9 days before parturition in B6 mice, and the capacity for induction increases markedly around the time of birth to reach maximal levels at 1 to 3 weeks of age. AHH induction by MC does not develop in the D2 mouse at any age. We therefore conclude that, during the more than 60 years' existence of the D2 inbred mouse strain, there has evolved a stable mutation (to some advantage for the D2 animal) in which there is no response to the foreign compound MC. And the normal (wild-type) response, as seen in B6 mice, is to increase the level of an enzyme which in turn metabolizes the polycyclic hydrocarbon inducer, so that the hydrophobic xenobiotic may be converted to more polar intermediates and products which can be readily excreted from the cell and from the body.

Studies by several laboratories of more than 30 inbred strains (*4*) indicate that about two-thirds of the mouse strains are genetically "responsive" (i.e., AHH inducible by aromatic polycyclic compounds) like wild-type or B6 mice and about one-third is "nonresponsive" like the D2 strain. It appears that the mutation in nonresponsive mice involves a defective cytosol protein receptor (*4,13*) and that nonresponsive mice have the necessary regulatory and structural genes for AHH induction by very potent polycyclic aromatic inducers; this subject, however, is beyond the scope of this report.

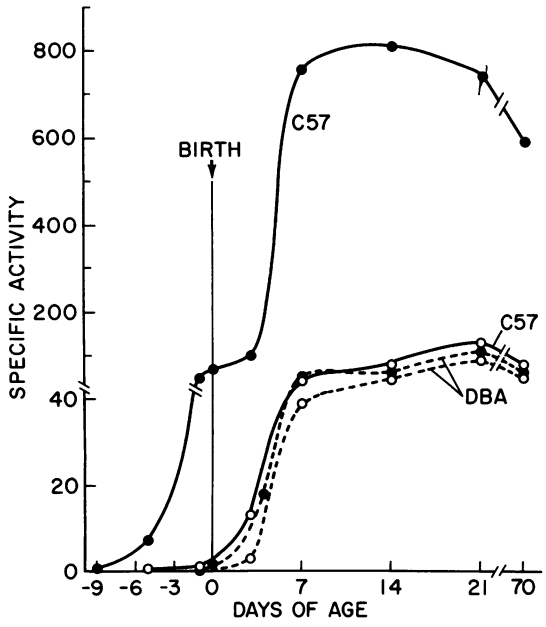

FIGURE 3. Hepatic levels of the basal aryl hydrocarbon hydroxylase activity and of the enzyme activity in response to MC treatment of C57BL/6N and DBA/2N mice, as a function of age (*11*): (●) mean hydroxylase specific activity from individual livers of 6-15 mice 24 hr after the intraperitoneal administration of 80 mg MC/kg of body weight; (o) mean enzyme activity from individual livers of 5-12 mice 24 hr after treatment with corn oil alone. The standard deviations of each group were always less than 25% and usually less than 15% of the mean specific activity. The closed circles depicting hydroxylase activity before birth represent the average specific activity found in five or more individual livers from a litter of fetuses whose mother had received intraperitoneally MC 24 hr. before; the closed circles on day "zero" indicate the mean enzyme activity from individual mice born within 24 hr after their mother had received the MC. Specific activity on the ordinate represents units per mg of total liver homogenate protein. A unit of AHH activity is defined (*9*) as that amount of enzyme catalyzing for 1 min at 37°C the formation of products the fluorescence of which is equivalent to 1 pmole of 3-hydroxybenzo[a]pyrene recrystallized standard.

Genetics of Aryl Hydrocarbon Hydroxylase Induction by Polycyclic Aromatic Compounds

AHH induction by MC is expressed almost exclusively as a single autosomal dominant trait (*9,11,14,15*) when B6 and D2 strains are used. When other strains are crossed (*4*) however, the simplest genetic model which can be offered involves a minimum of six alleles at a minimum of two genetic loci. For our genetic experimental model system, we therefore routinely use offspring from the appropriate crosses between B6 and D2 parent strains: Ah^b is the dominant allele for responsiveness; Ah^d is the recessive allele, the Ah^d/Ah^d animal being genetically nonresponsive.

The autosomal dominant trait for AHH induction by polycyclic hydrocarbons was found to be expressed more or less dominantly in nonhepatic tissues as well (*4,14*). A careful dose-response curve of AHH inducers (*4*), however, indicates that the amount of induced AHH activity in liver, kidney, bowel, or lung in the B6 mouse is always slightly greater than that in the (B6D2)F_1 heterozygote and is always considerably greater than that in the D2 mouse. Similar results exist in skin (*14,16*), lymph nodes (*16*), bone marrow (*17*), and the pigmented epithelium of the retina (*18*), although differences in inducible AHH activity in these tissues between B6 and D2 mice are often not as striking as those in bowel, kidney, and liver. The fact that the magnitude of AHH and cytochrome P_1-450 induction by polycyclic aromatic compounds appears to be genetically regulated in most tissues of the mouse (*4,5*) is of importance for the remainder of this report.

Differences in Metabolites Produced by Different Forms of Cytochrome P-450

Several lines of evidence indicate that at least two different AHH activities exist (*4*) and are associated with different forms of P-450: the enzyme from MC-treated responsive mice associated with cytochrome P_1-450; and the enzyme from control or phenobarbital-treated responsive and nonresponsive mice, or from MC-treated nonresponsive mice, associated principally with some form(s) other than P_1-450. This finding is extremely important, because it is now apparent that different forms of P-450 may generate different ratios of metabolites from the same substrate. Comparing MC versus phenobarbital as the inducer in rat liver, for example (Fig. 4), various groups have shown that hydroxylations may occur predominantly in different chemical positions on the molecule for such substrates as biphenyl (*19*), testosterone (*20*), 2-acetylaminofluorene (*21,22*), bromobenzene (*23*), *n*-hexane (*24*), and benzo[a]pyrene (*25,26*). Such differences in the metabolite profile of a polycyclic hydrocarbon or other foreign chemical reflect presumed differences in the nature of the intermediates formed; differences in the reactivity of these intermediates might result in marked dissimilarities in the toxicity or carcinogenicity of a given compound. A good example is the metabolism of

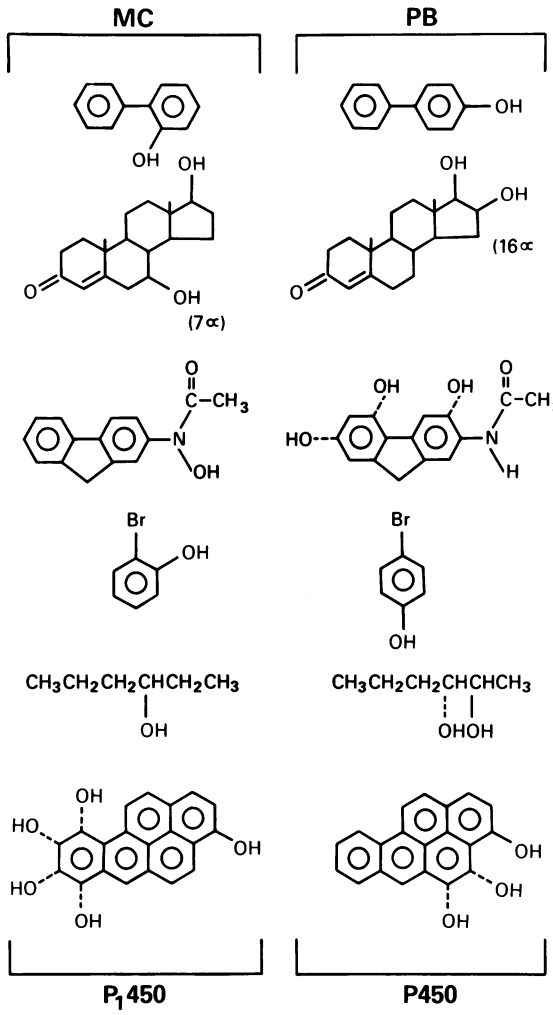

FIGURE 4. Chemical structures of known differences in metabolite formation (*7*) when each of these six substrates is oxygenated *in vitro* with liver microsomes from rats treated with MC or phenobarbital (PB). Similar differences in metabolite profile exist in mice for biphenyl (*27*), 2-acetylaminofluorene (*28*), and benzo[a]pyrene (*29*), but not for testosterone (*30*) or bromobenzene (*31*). To our knowledge n-hexane metabolites have not been examined in MC- and phenobarbital-treated mice.

bromobenzene in rats. The *p*-phenolic derivative of bromobenzene, presumably arising from the 3,4-oxide, is associated in some manner with hepatic necrosis, whereas the *o*-phenolic derivative, presumably arising from the 2,3-oxide, is not (*23*). This finding is an example (in rats) in which the higher amounts of cytochrome P_1-450 are beneficial to the animal.

With a similar goal in mind, members of this laboratory for the past several years have searched for differences in drug metabolism, toxicity, and susceptibility to cancer which can be shown to be associated with differences in a single gene (or small number of genes). Although tumorigenesis initiated by DMBA is not associated with the *Ah* locus, for example, subcutaneous sarcomas initiated by MC are associated with inducible AHH activity among 14 inbred strains of mice (*32*). With the use of offspring from the F_1 to parent backcrosses and the $F_1 \times F_1$ intercross, MC-initiated tumorigenesis was shown recently (*33*) to be highly correlated with the Ah^b allele.

The remainder of this report deals with two examples of developmental toxicity which appear to be associated with the Ah^b allele: birth defects produced by MC and DMBA; and acetaminophen-produced glutathione depletion and hepatic necrosis, appearing postnatally.

Differences in *Ah* Locus Found *in Utero*

Figure 5 shows that the genetically mediated AHH induction by MC can be present or relatively absent among different fetuses in the same uterus. In the experiment illustrated, the enzyme in seven out of ten (B6D2)F_2 fetuses in the same uterus of a MC-treated (B6D2)F_1 mother is about 5 to 15 times greater than that found in the placenta, fetal bowel, or fetal liver of the three nonresponsive individuals or of control F_2 mice. If AHH activity is increased in the placenta the enzyme is also increased in fetal liver and bowel of that individual, and vice versa. The fact that the responsive mother or responsive individuals in the uterus do not influence AHH induction in the nonresponsive fetuses or their placentas in the same uterus indicates that no humoral agent circulating in the pregnant animal is able to "derepress" AHH induction in Ah^d/Ah^d fetuses.

This experiment caused us to wonder whether certain birth defects could be explained on the basis of genetic predisposition of a particular fetus rather than genetic predisposition of the mother. Such an hypothesis could explain why a birth defect is found in one child, for example, when the mother has received the same dose of the same anticonvulsant during each of six pregnancies. We first tested this hypothesis with polycyclic hydrocarbons in responsive and nonresponsive strains, since these chemicals are known to be toxic and are known to be metabolized by one or more forms of cytochrome P-450.

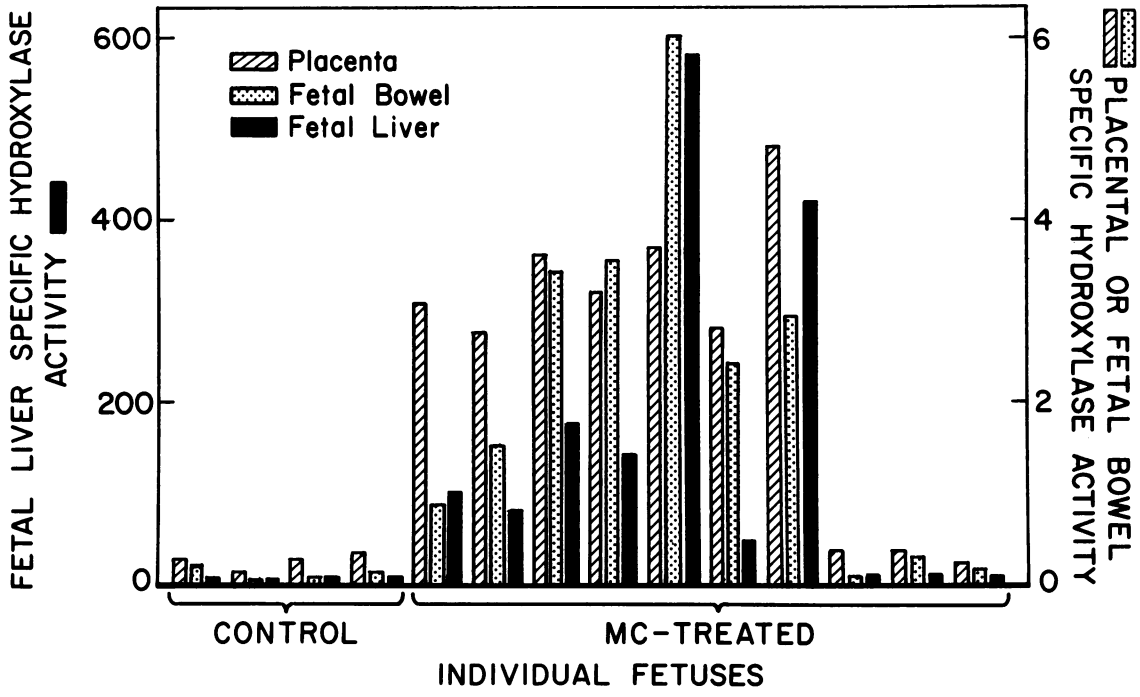

FIGURE 5. Placental, fetal bowel, and fetal liver aryl hydrocarbon hydroxylase activities in (B6D2)F_2 fetuses from a MC-treated (B6D2)F_1 mother (*34*). MC was administered intraperitoneally (80 mg/kg) at about 19 days of gestational age, and enzyme activities on individual fetal mice were determined 24 hr later. A control (B6D2)F_1 at about 19 days of gestation received corn oil alone. Specific hydroxylase activities are expressed as units/mg of tissue homogenate protein for placenta and fetal bowel and units per mg of microsomal protein for fetal liver.

Association of *Ah* Responsiveness with Teratogenesis Produced by MC and DMBA

Table 1 shows that, among the 35 litters in control mice, no malformations are observed and between 6.9 and 12.5% stillborns and resorptions are seen among the four inbred strains. These values are in line with other studies (*36,37*) using similar inbred mouse strains; for example, 0.05% of 1947 C57BL/10Gn newborns was polydactylous (*37*). The combination of stillborns, resorptions, and malformations can be seen to be generally greater among MC- or DMBA-treated responsive B6, C3H/HeN, and BALB/cAnN, compared with that in MC- or DMBA-treated nonresponsive AKR/N (AKR). We repeatedly attempted to breed DBA/2N, 129/J, and AU/SsJ—three other nonresponsive inbred strains—but the rates of pregnancy were too low to be included in these teratology studies. Except for one malformation (pigmented lesion on forehead) in DMBA-treated AKR, all malformations—a total of 21—are found among 51 litters of polycyclic hydrocarbon-treated B6 mice.

Table 2 summarizes all the data, regardless of the dose of MC or DMBA, for the day of gestation on which the chemical was administered. We realize that the number of letters of responsive versus nonresponsive strains is not the same at each dose of MC or DMBA given (Table 1); in spite of this, comparing all responsive with nonresponsive mice receiving MC or DMBA (Table 2) provides us with valuable information. The incidence of malformations in responsive mice, compared with that in nonresponsive mice, is striking: 6.5% to 0% for MC; 4.6% to 0.4% for DMBA. The combined stillborns, resorptions, and malformations are 2- to 3-fold greater in responsive than in nonresponsive mice for both MC and DMBA: 47% to 18% for MC; 28% to 11.5% for DMBA. If the background defects (in control animals) are subtracted from the experimental data, the combined stillborns, resorptions, and malformations are 5- and about 20-fold greater for MC- and DMBA-treated responsive mice, respectively, compared with corresponding nonresponsive mice: 39% to 7.6% for MC; 20% to 1.1% for DMBA.

Table 1. Developmental toxicity of MC and DMBA among several inbred strains of pregnant mice.[a]

Test compound	Dose, mg/kg	Day of gestation	Inbred strain[b]	Number of litters	Number of implantations	Stillborns	Resorptions	Malformations		SRM, %[c]	Malformations, %
								Club foot	Other		
None			B6	12	116	8	0	0	0	6.9	0
			C3H	4	16	0	2	0	0	12.5	0
			BALB	3	18	2	0	0	0	11.1	0
			AKR	16	134	14	0	0	0	10.4	0
MC	70	5	B6	4	13	0	13	0	0	100	
			AKR	12	104	6	16	0	0	21	0
		7	B6	6	41	0	29	3	0	78	7.3
			AKR	2	16	0	2	0	0	12.5	0
		10	B6	5	32	1	4	3	0	25	9.4
			C3H	3	20	0	0	0	0	0	0
			AKR	8	49	0	4	0	0	8.2	0
		12	B6	4	32	0	9	2	1[d]	38	9.4
			AKR	5	49	1	11	0	0	24.5	0
DMBA	50	11	B6	1	6	0	2	1	0	50	17
			AKR	1	2	0	1	0	0	50	0
		12	B6	4	34	1	9	2	1[e]	38	8.8
			C3H	2	15	0	5	0	0	33	0
			AKR	11	46	0	7	0	0	15	0
	25	12	B6	23	140	0	23	8	0	22	5.7
			C3H	3	30	2	4	0	0	20	0
			BALB	2	15	0	7	0	0	47	0
			AKR	14	105	0	13	0	0	12	0
	50	13	B6	4	19	0	8	0	0	42	0
			AKR	10	72	0	4	0	1[f]	7.0	1.4

[a] Evidence of a vaginal plug the morning after mating was the criterion for conception (*35*), and this was designated day 1 of gestation. MC or DMBA in corn oil was administered intraperitoneally at the indicated doses and about 9 A.M. on the days of gestation shown. Animals not receiving the test compound received corn oil alone at the same dose (2.5 ml/kg body weight). The pregnant mothers were killed on day 18 of gestation, and the contents of the uterus were examined.

[b] B6, C3H, BALB, and AKR denote C57BL/6N, C3H/HeN, BALB/cAnN, and AKR/N inbred strains examined in 1974 and 1975.

[c] Combined stillborns, resorptions, and malformations, compared with the total number of implantation.

[d] Unilateral anophthalmos.

[e] Curled tail.

[f] Pigmented lesion on forehead.

Table 2. Summary of genetic differences in MC- and DMBA-induced developmental toxicity in mice.

Test compound	Aromatic hydrocarbon responsiveness phenotype	Number of litters	Number of implantations	Stillborns	Resorptions	Malformations		SRM, %[a]	Malformations %
						Club foot	Other		
None	Responsive	19	150	10	2	0	0	8.0	0
	Nonresponsive	16	134	14	0	0	0	10.4	0
MC	Responsive	22	138	1	55	8	1	47	6.5
	Nonresponsive	27	218	7	33	0	0	18	0
DMBA	Responsive	39	259	3	58	11	1	28	4.6
	Nonresponsive	36	225	0	25	0	1	11.5	0.4

[a] Abbreviations are the same as in Table 1.

With the results so far, however, we are comparing inbred strains only. A similar study, for example, showed a significantly greater incidence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced kidney anomalies in C57BL/6J fetuses than in DBA/2J fetuses (*38*). Experiments using MC- and DMBA-treated pregnant mice with (B6D2)D2 or (B6AKR)AKR individuals *in utero* are currently being performed. Only after malformations are shown to be associated with the responsive phenotype can we conclude that the Ah^b allele, rather than merely a difference between inbred strains, is associated with polycyclic hydrocarbon-induced teratogenesis. Other hydrophobic chemicals and drugs—known or suspected to interact with one or more forms of P-450—are also being tested in this model system.

Acetaminophen Metabolism

Various other monooxygenase "activities" are associated with AHH induction by polycyclic hydrocarbons and therefore the Ah^b allele and cytochrome(s) P_1-450: *p*-nitroanisole *O*-demethylase, 7-ethoxycoumarin *O*-deethylase, 3-methyl-4-methylaminoazobenzene *N*-demethylase, zoxazolamine 6-hydroxylase, 2-acetylaminofluorene *N*-hydroxylase, phenacetin *O*-deethylase, naphthalene monooxygenase, acetanilide 4-hydroxylase, biphenyl 4-hydroxylase, and biphenyl 2-hydroxylase (*4*). It now appears that the *N*-hydroxylation of acetaminophen (Fig. 6), like that of 2-acetylaminofluorene, is predominantly mediated by P_1-450; the *N*-hydroxylation of several other *N*-acetylarylamines also appears to be catalyzed by P_1-450 (*40*). The rearrangement of the *N*-hydroxy derivative to a highly reactive electrophile has been postulated (*39*) to be the principal mechanism by which glutathione or covalently bound protein or nucleic acid occurs *ortho* to the hydroxyl group. The amount of radioactive metabolite covalently bound to acid-precipitable material, following administration of large doses of [^{3}H]acetaminophen, is associated in the hamster and mouse with gluthathione depletion and the magnitude of hepatic necrosis observed (*39*). It recently has been found (*28,40*) that the Ah^b allele is highly correlated with acetaminophen-induced hepatotoxicity and increases in covalently bound metabolites of the drug.

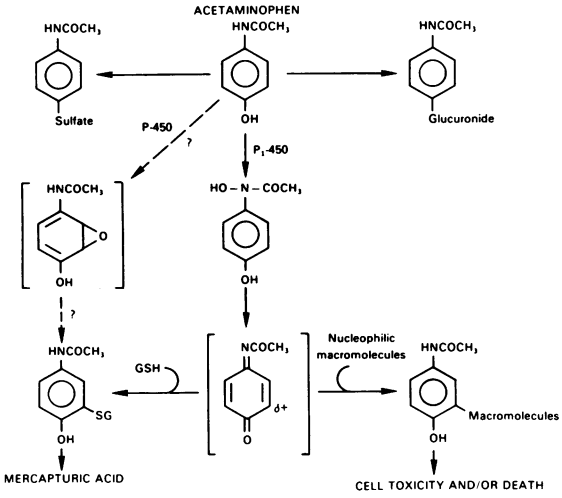

FIGURE 6. Known and suspected metabolic pathways for actaminophen. The dashed arrows with question marks represent pathways that have not been substantiated experimentally, and the compounds in brackets are postulated intermediates modified from Potter (*39*).

Hepatic Glutathione Depletion Caused by Acetaminophen

Figure 7 demonstrates the developmental curve for total liver glutathione content in mice. The concentration of oxidized glutathione is

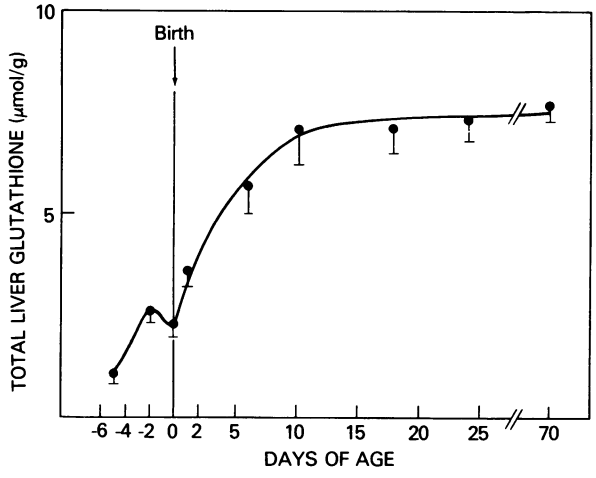

FIGURE 7. Ontogenetic expression of total hepatic glutathione content in mice (*41*). Oxidized plus reduced glutathione were determined by the method of Tietze (*42*). As there were no significant differences between B6 and AKR mice, the values from both inbred strains are included. In this figure and in subsequent figures, the symbols and brackets represent means ± standard errors for individual determinations. Five to 20 mice were tested at each age in this experiment.

always between 2% and 6% of the total content in this figure and in subsequent figures (*41*). Total glutathione content in fetal liver is quite small when compared with that in adult liver, increases at birth to about 33% of adult levels, and reaches adult levels at about 10 days postpartum. Similar developmental curves—such as this for glutathione content and Fig. 3 for basal and inducible AHH activity—are common for numerous enzymes, e.g., γ-glutamyltransferase (*43*), tyrosine aminotransferase (*44*), glucose-6-phosphate dehydrogenase (*45*), uridine kinase (*46*), lysyl hydroxylase (*47*), and pyruvate decarboxylase (*48*).

It can be seen in Figure 6 that an increase in *N*-hydroxylation enhances the need for reduced glutathione. It is known (*39*) that glutathione depletion precedes marked increases in covalently bound acetaminophen. Figure 8*A* shows glutathione depletion in maternal liver following acetaminophen administration. Glutathione content decreases to about 15% of normal 2 hr after a 250 mg/kg dose but returns to 63% and 120% of normal 3 hr and 5 hr, respectively, after the drug was given. Twenty-four hours later the glutathione content is 165% of normal; the mechanism for this overcompensation is not understood. Both 500 and 1000 mg/kg of acetaminophen depletes glutathione content to less than 10% of normal within 2 hr, and there is no recovery during the next 3 hr. In most instances these pregnant mice die within 12 hr, the cause of death presumably related to the irreparable hepatic necrosis at these large doses of acetaminophen. In contrast to the results in maternal liver, no significant glutathione depletion in fetal liver occurs after the 250 mg/kg dose of acetaminophen (Fig. 8*B*), and there is only about 50% depletion 5 hr after the 1000 mg/kg dose.

The reasons for this striking "protection" of the fetoplacental unit against the toxic effects of acetaminophen are not known. Acrylamide, at doses which produce neuropathy in pregnant rats, fails to cause adverse effects in fetuses near term; this protection of the fetoplacental unit was shown not to be due to failure of acrylamide to cross the placenta (*49*). Likewise, acetaminophen crosses the placenta readily (Fig. 9).

Figure 10 illustrates that liver glutathione in mice 10 days of age or less in relatively resistant to depletion by acetaminophen. By 15 days of age, however, the glutathione depletion is as great as that seen in the adult. There may be a greater rate of sulfate or glucuronide conjugation, relative to the P_1-450 N-hydroxylation pathway (see

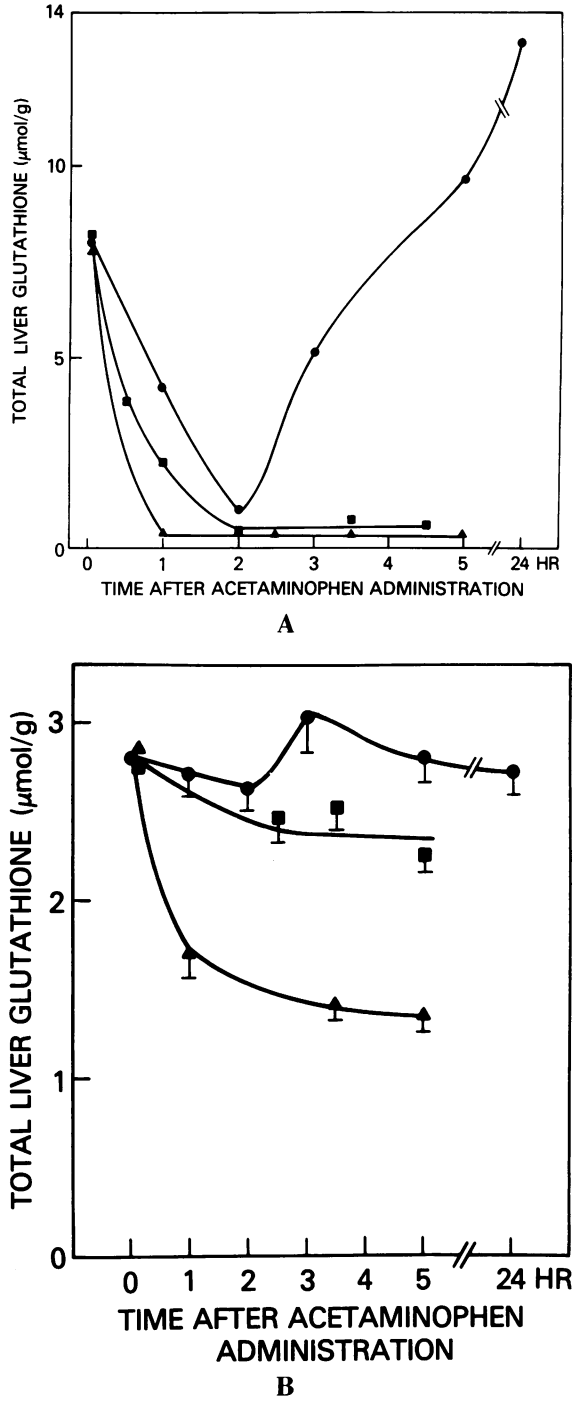

FIGURE 8. Rate of total glutathione depletion in (*A*) maternal liver and (*B*) fetal liver following the intraperitoneal administration of varying doses of acetaminophen to AKR mice of 18 days gestational age (*41*): (●) 250 mg/kg; (■) 500 mg/kg; (▲) 1000 mg/kg. Each symbol represents the value from four combined livers.

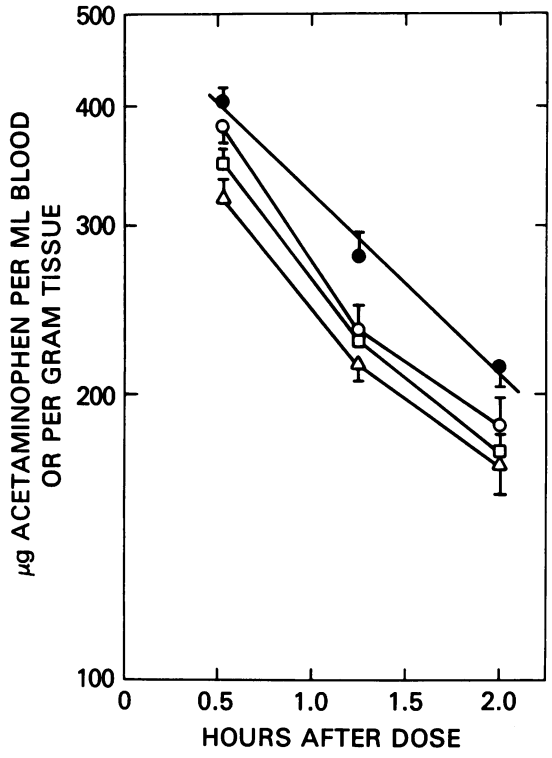

FIGURE 9. Rates of disappearance of acetaminophen from (●) maternal blood, (o) maternal liver, (△) fetal liver, and (□) placenta, following administration of 500 mg of drug per kg to an AKR mouse of 18 days gestational age (*41*). [^{3}H]acetaminophen (final specific radioactivity of 0.9 μCi/μmole) was given, and the parent drug that remained at various time intervals was determined according to the method of Mitchell et al. (*50*). Three mothers, two littermates and two placentas from each mother were used in this experiment.

Fig. 6) in the liver of mice 10 days of age or less, compared with that in older mice.

Covalent Binding of Acetaminophen Associated with the *Ah*^{b} Allele

Figure 11 shows that MC treatment enhances glutathione depletion by acetaminophen in both maternal and fetal liver of genetically responsive mice. Such an enhancement is not seen in pregnant nonresponsive AKR/N mice. These data suggest that the *N*-hydroxylation of acetaminophen is associated with P_1-450 induction and therefore the *Ah*^{b} allele. This suggestion was recently confirmed (*40*); using MC-treated progeny from the appropriate backcrosses and intercross of the B6 and D2 strains, Wirth and coworkers demonstrated a highly statistically significant correlation between the *Ah*^{b} allele and increased covalent binding of acetaminophen metabolites to acid-precipitable material.

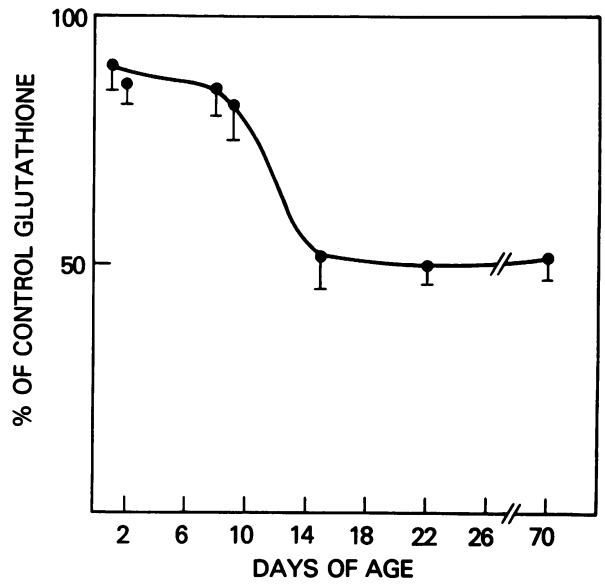

FIGURE 10. Ontogenetic expression for hepatic total glutathione depletion in AKR mice which had been treated for 30 min with intraperitoneal acetaminophen (500 mg/kg) (*41*). Individual determinations on three acetaminophen-treated and three control mice at each age were compared.

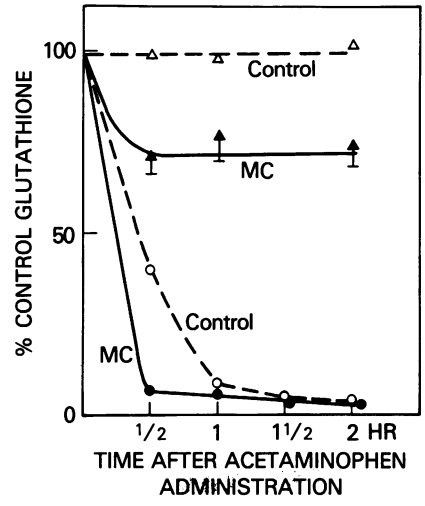

FIGURE 11. Total tissue glutathione content in mice of 18 days gestational age following a single dose of acetaminophen (500 mg/kg) (*41*): (●,▲) B6 mice treated with MC 48 hr before the experiment; (O, △) represent control B6 and AKR mice. For each time point, (●, O) two maternal livers or (▲, △) three to five fetal livers were combined for glutathione determinations and plotted as per cent of control. The starting level of control glutathione in this experiment was 7.8 μmole/g liver.

Pathological Conditions Associated with Ah^b Allele or Aromatic Hydrocarbon Responsiveness

Table 3 summarizes the various effects caused by differences in xenobiotic metabolism due to the *Ah* locus, which have been reported by this laboratory as well as by Kouri, Hutton, et al. DMBA-produced skin inflammation, MC-or DMBA-caused birth defects, benzo[a]pyrene-initiated tumors, and acetaminophen-caused cataracts all have been studied to date only as differences among inbred strains. All other effects listed in Table 3 have been demonstrated to be correlated specifically with the Ah^b allele by means of studies using siblings from the (B6D2)F_1 × D2 backcross. *In vitro* effects that have been shown to be correlated with the Ah^b allele include MC, 6-aminochrysene, and 2-acetylaminofluorene mutagenesis (*55*) and differences in the profile of benzo[a]pyrene metabolites binding to DNA nucleosides (*7*).

Table 3. Conditions in the mouse associated with the Ah^b allele or with aromatic hydrocarbon responsiveness.

Condition	References
Increased susceptibility to MC-initiated subcutaneous sarcomas	(*32, 33*)
Increased susceptibility to 7,12-dimethylbenz[a]-anthracene-produced skin inflammation	(*51*)
Shorter zoxazolamine paralysis time	(*52*)
Increased susceptibility to acetaminophen-caused hepatic necrosis	(*28, 40, 41*)
Shorter survival time when given large doses of polycylic aromatic compounds or polychlorinated biphenyls intraperitioneally	(*31*)
Increased resistance to lindane given intraperitoneally	(*31*)
Increased resistance to polycyclic hydrocarbons or lindane given orally	(*31*)
Increased resistance to aplastic anemia caused by oral benzo[a]pyrene	(*17, 31*)
Shorter survival time when given polychlorinated biphenyls orally	(*31*)
Increased susceptibility to stillborns, fetal resorptions, and malformations caused by MC or 7,12-dimethylbenz[a]anthracene given to pregnant mother	This report
Increased susceptibility to benzo[a]pyrene-initiated subcutaneous sarcomas	(*7, 53*)
Increased susceptibility to squamous cell carcinomas of the lung initiated by intratracheal instillation of MC	(*53*)
Increased susceptibility to cataract formation caused by acetaminophen given intraperitoneally	(*54*)

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